Fast Tracking Biotherapeutic Development via Flow Electroporation™ Technology for Large Scale Transient Expression and Cell Line Generation Using Your CHO Cell Line of Choice.

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Abstract

Companies are turning to transient production of antibodies during early development to delay stable cell line generation, accelerate timelines, and reduce costs. A key factor for the success of this approach is the production of high quality antibodies with a high degree of similarity to stably produced proteins. MaxCyte's flow-electroporation based delivery platform offers significant advantages across the therapeutic development pipeline by transiently producing milligram to gram quantities of protein and rapidly generating stable pools and stable clones using cell backgrounds that are relevant to biomanufacturing. In this poster, data are shown including transfection performance in multiple CHO cell lines as well as protein quality and glycosylation profiles that demonstrate the similarity of transiently stably produced antibodies. Furthermore, we demonstrate that the high levels of transfection efficiency and cell viability post electroporation can significantly reduce the time needed for cell recovery during selection and create stable pools enriched for high producers. These features shorten the timelines and reduce the labor needed for creating clonally-derived cell lines. In summary, we illustrate that MaxCyte Flow Electroporation™ Technology can expand the footprint of transient transfection by delaying stable cell line generation while maintaining the integrity of candidate selection thereby accelerating early to mid-stage biotherapeutic development.

High Performance Transfection of Multiple CHO Cell Lines

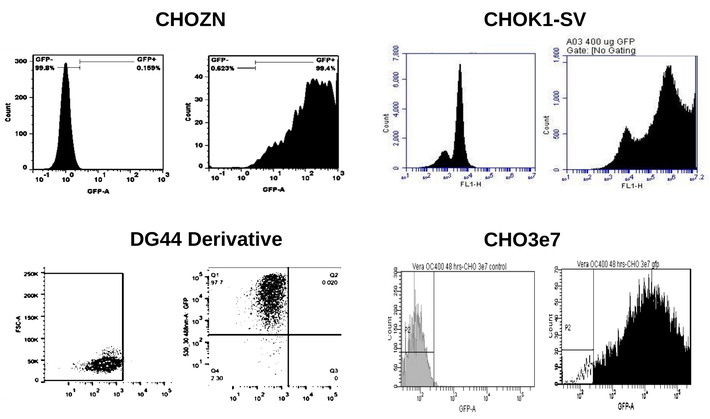
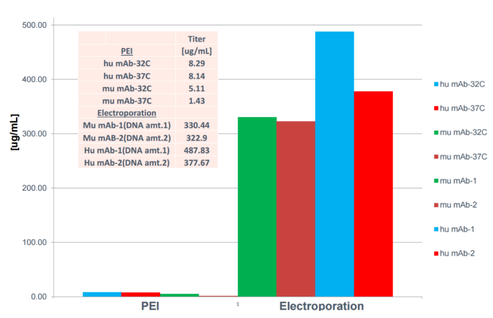


Figure 1: High Performance Transfection of a Variety of CHO Cell Lines. CHOZN, CHOK1-SV, CHO3e7 and CHO DG44-derived cells were transfected with a GFP expression plasmid via small scale electroporation on the MaxCyte STX®. FACS analysis was performed 24-48 hrs post electroporation.

MaxCyte Outperforms PEI of CHO-K1SV Cells

Large Increase in Mouse & Human IgG Expression



PEI	Titer [ug/mL]
hu mAb-32C	8.29
hu mAb-37C	8.14
mu mAb-32C	5.11
mu mAb-37C	1.43
Electroporation	
Mu mAb-1(DNA amt.1)	330.44
Mu mAB-2(DNA amt.2)	322.9
Hu mAb-1(DNA amt.1)	487.83
Hu mAb-2(DNA amt.2)	377.67

Figure 2: PEI vs. MaxCyte Comparison for Antibody Expression in CHO-K1SV Cells. CHO-K1SV were transfected using PEI or the MaxCyte STX for a human or mouse IgG. Two [DNA] were tested using MaxCyte electroporation. All MaxCyte transfected cells were shifted to 32°C post electroporation. Half of PEI samples were shifted to 32°C while half remained at 37°C post transfection. Transfection efficiency was determined by antigen binding/ titer ELISA of transfection conditioned media. Antibody titers of day 9 media were determined for all transfection and post transfection conditions. Inset table shows actual antibody titer levels depicted in the associated graph.

CHO EBNA & 293 EBNA: PEI versus MaxCyte

Superior Antibody Expression Using the MaxCyte STX

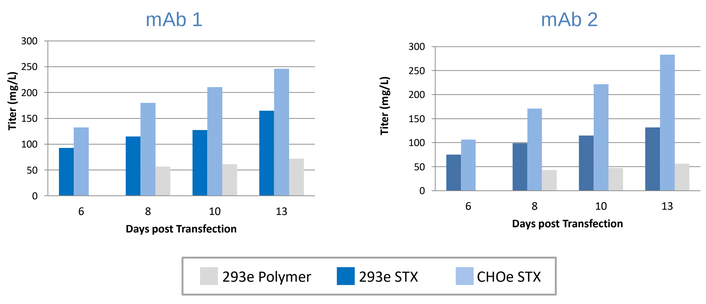


Figure 3: High Titer mAb Expression in CHO EBNA and 293 EBNA Cells. CHO EBNA and 293 EBNA cells were transfected with an IgG expression plasmid via static electroporation (6E7-8E7 cell per condition) and cultured in 125 mL shake flasks for 13 days. Secreted antibody titers in both STX-transfected cell lines greatly exceeded titers generated by an optimized PEI transfection method of 293 EBNA cells.

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CHOZN®: Gel Analysis of Protein Quality & Titer

50Kda Heavy Chain and the 25Kda Light Chain Are Clearly Seen

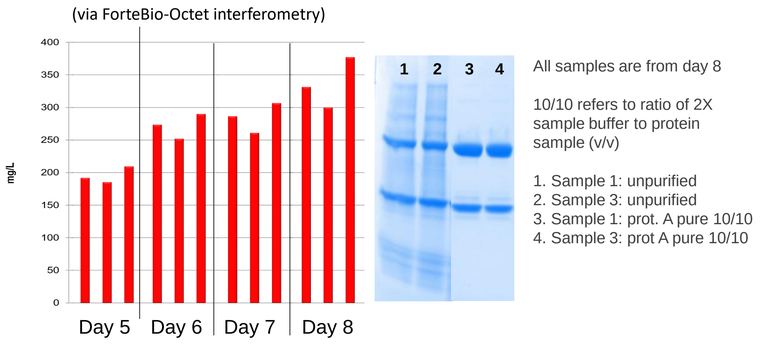


Figure 4: Titers & SDS PAGE analysis of transiently expressed IgGs. CHOZN® cells were electroporated using the MaxCyte STX – three independent runs. Conditioned media samples were collected on days 5-8 and analyzed for IgG titers. Samples from day 8 post EP were run on a Novex 4-20% SDS PAGE Tris Glycine gel and stained with Coomassie Blue G-250. Bands of the correct size for hIgG heavy and light chains are clearly evident on a reducing gel loaded with unpurified media samples and Protein A purified samples. No additional bands are evident in the purified samples, indicating good protein quality.

Multi-Gram, CHO-S Antibody Production Following Feed Optimization

> 2.7 g/L Antibody Titers in <3 Weeks

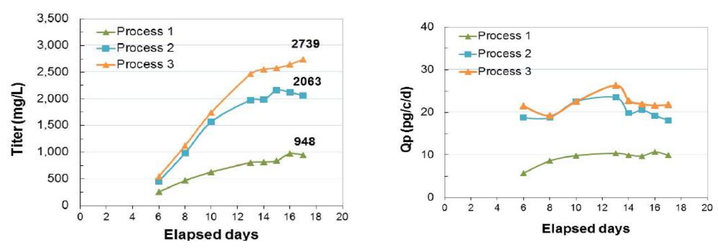


Figure 5: Post Electroporation Culture Process Optimization Produces CHO-S Antibody Titers >2.7 g/L. CHO-S cells were transfected with an antibody expression plasmid (1µg DNA/1E6 cells) via small scale electroporation on the MaxCyte STX®. Cells were plated at approximately 4E6 cells/mL post electroporation. Transfected cells were cultured in media with different additives and culture conditions. Titer was verified by both ELISA and Protein A capture assays. The optimized process produced antibody titers of 2.7 g/L at day 17 post electroporation as a fed batch.

Consistent Antibody Quality & Glycosylation

Similar IgGs Produced via MaxCyte Transient Transfection & Stable Cell Lines Generated Using MaxCyte Transfection

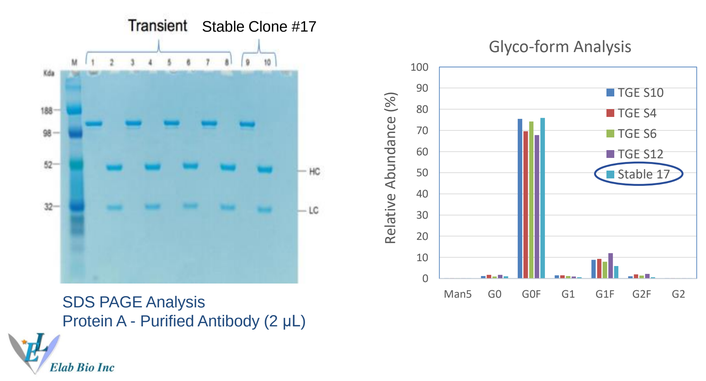
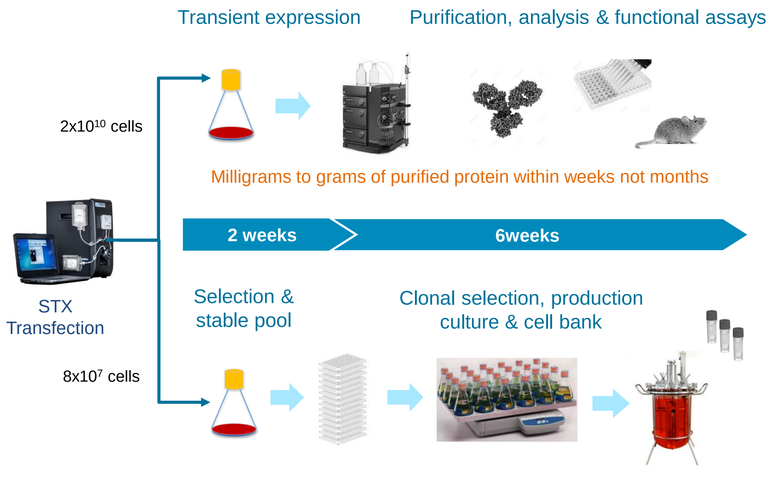


Figure 6: hIgG1 Quality & Glyco-form Comparison - Transient vs Stable Expression. A human IgG molecule was expressed transiently in CHO-S cells via four independent electroporation runs on the MaxCyte STX. A stable cell line (S17) was also generated by subjecting transfected cells to antibiotic selection, followed by limited dilution cloning. A). Glycoform analysis showed highly consistent patterns of post-translational modification between transiently and stably proteins. B.) SDS-PAGE gel analysis (reducing and non-reducing) data indicate equivalent quality (i.e aggregation or degradation) of antibodies produced via transient or stable transfection.

Simultaneous Transient Expression & Stable Generation



Rapid CHO Cell Recovery

7 Days Faster than Chemical-based Transfection

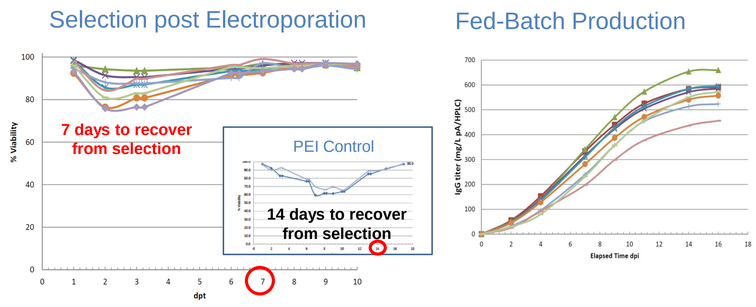
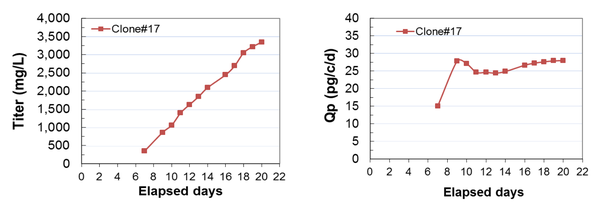


Figure 7: Rapid Recovery of Stable Pools Following MaxCyte Electroporation. CHO cells (proprietary cell line) were transfected via static electroporation in OC-400 processing assemblies with varying concentrations of an antibody expression plasmid. Transfected cells were cultured in shake flasks and were subjected to MSX selection beginning on the day of electroporation. STX transfected cells recovered from selection 7 days quicker than compared to cells transfected with the same plasmid using PEI.

Rapid, High-Yield Stable Cell Generation

Stable Cell Lines Generated in 6 Weeks

A. High-Producing Stable Clone Identified



B. Process Development Boosts Stable Clone Production

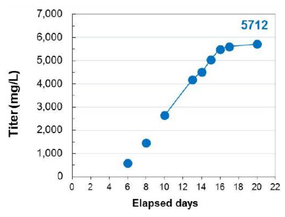


Figure 8. Rapid Stable Cell Line Development Using MaxCyte Flow Electroporation. A stable pool of CHO cells expressing an hIgG was generated within two weeks of electroporation. A). 479 clones were screened following limited dilution cloning. The top clone (clone #17) was selected for production within 6 weeks post transfection. B). The production culture was carried out in shake flasks as a fed batch. At day 17, productivity reached >5.5 g/L. Results were verified by both ELISA and Protein A capture assays.

Summary

- The MaxCyte delivery platform consistently results in high transfection efficiency for a variety of CHO cell lines providing researchers the flexibility to use the CHO cell line of choice throughout their biotherapeutic development activities.
- MaxCyte Flow Electroporation™ Technology outperforms other transfection methods including chemical-based methods such as PEI resulting in higher, more consistent antibody production.
- CHO cell lines exhibit strong human and mouse IgG production following MaxCyte electroporation. CHO-S cells exhibited titers >2.7g/L following feed optimization enabling rapid, gram-scale production of antibodies.
- Antibodies transiently produced using MaxCyte electroporation demonstrate similar protein characteristics and glycosylation patterns to antibodies produced by stable cell lines, supporting the use of transiently produced proteins in early-stage discovery efforts allowing the delay of stable cell line generation and thereby reduced costs.
- MaxCyte electroporation is fully scalable allowing a single large-scale production for transient protein production and simultaneous generation of stable clones.
- The high cell viability and transfection efficiencies of MaxCyte's delivery platform generate quality stable pools faster than chemical-based methods.
- High CHO cell viability post electroporation enables rapid generation of stable pools and development of stable clones in 6 weeks with yields >5.7 g/L. High cell viability enabled stronger antibiotic selection which enriches for high yield clones. Fewer than 500 clones needed to be screened to identify a high-yield stable cell line.

MaxCyte Delivery Platform for Cell Engineering

The MaxCyte STX® and MaxCyte VLX® Transfection Systems use fully scalable Flow Electroporation™ Technology for rapid, highly efficient, highly consistent transfection.

MaxCyte STX®
5E5 Cells in Seconds
Up to 2E10 Cells in <30 min

MaxCyte VLX®
Up to 2E11 Cells in <30 min

- High efficiency & high cell viability
- Broad cell compatibility
- True scalability requiring no re-optimization
- Closed, computer-controlled instruments
- cGMP-compliant & CE-marked system with FDA Master File